TITLE:

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CITATION:

MITSUDA, HISAKAZU. NEUROHISTOLOGICAL STUDY ON THE NERVE STRUCTURES IN THE APPENDIX OF HUMAN BEING WITH THE TARTARIC ACID-THIONIN ENCLOSURE METHOD. 日本外科宝函 1960, 29(2): 407-413

ISSUE DATE:

1960-03-01

URL:

http://hdl.handle.net/2433/207094

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NEUROHISTOLOGICAL STUDY ON THE NERVE STRUCTURES IN THE APPENDIX OF HUMAN BEING WITH THE TARTARIC ACID-THIONIN ENCLOSURE METHOD

by

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Received for publication Dec. 8, 1959

I. INTRODUCTION

One of the most complicated problems in the peripheral nerve is the structure of the terminal network of the autonomic nerve fibers. BOEKE (1935) detected the fine nerve network with free terminals in the effector tissue cells and called it sympathetic fundamental plexus (sympathische Grundplexus), PH. STOEHR jr. supposed that the peripheral nerve fibers form a closed network with or without accompanied by the SCHWANN's cell plasmodia and he maintains the continuity of nerve element between pre- and postganglionic fibers, which lead the conclusive denial in the existence of synapsis as well as the reality of neurontheory.

HERZOG, WEEER, JABONERO and others have strong opposition against STOEHR. They affirm the synapsis and deny the terminal network not enclosed in the SCHWANN's cell plasmodia.

Recently YAMAMOTO of our laboratory observed the nerve structure in the appendix of human being with the use of electron microscope and reported a noticeable results that the autonomic nerve fibers lose the SCHWANN's membrane only in the unstriated muscles, i. e. at their ending. According to YAMAMOTO the nerve fibers always ran through a canal made of SCHWANN's membrane or Axon-SCHWANN membrane and not through the SCHWANN's cell protoplasm.

As for the SCHWANN's plasmodium YAMAMOTO recognized the clear borderings between adjacent cells and denied the structure of syncitium. According to him the terminal network structure in the nervous syncitium by JABONERO represents a group of unmyelinated nerve fibers running through a common Axon-SCHWANN membrane.

FEYRTER described the shortage of silver impregnation method in the study of the terminal network of the autonomic nerves because of the indefinite figures given by this method. He maintains the necessity of the critical study by other method and recommends the use of tartaric acid-thionin enclosure staining for the observation of the nervous syncitium. According to FEYRTER the nervous syncitium and unmyelinated fibers is stained in light red, myelinated fibers in bright red and the nerve cells in blue.

Recently M. OKADA of our laboratory presented a clear features of nerve structures in human appendix by means of silver impregnation, the author, in the light of OKADA's report, planed to study the nerve structures mainly of AUERBACH's

plexus with the use of tartaric acid-thionin enclosure staining by FEYRTER.

Ⅱ. MATERIALS AND METHODS

Fresh human appendices were used for staining. The original method by FEYRTER was followed.

1. The materials were fixed in 10% neutral formol solution for 24 hours.
2. Washed in water for 15 minutes.
3. Sliced with freezing microtome and were washed with destilled water.
4. Sections were stretched on the object glass.
5. Tartaric acid-thionin solution (1.0 g of thionins, 0.5 g of tartaric acid were dissolved in 100 cc of destilled water) was dropped on the specimen.
6. Covered, and the water on the edges of the cover was removed as rapidly as possible.
7. The edges were enclosed with wax.

Above mentioned original method by FEYRTER gave strong red tone covering the whole tissue and made the figures in distinct, so that the author fixed the material for 48 hours in the climate of spring and autumn (18～20°C) and 24 hours in summer (25～30°C). The section had a thickness about 30μ. The tartaric acid-thionin solution could be used for about 10 days after preparation. The suitable time of dropping of the solution was 5 minutes in spring and autumn, and 2 minutes in summer. The tone of the tissue colours under cover changed in 2 weeks in spring and autumn, and in 1 week in summer and the observation became no longer possible.

Ⅲ. FINDINGS

1) The figure of a ganglion in the sympathetic trunk stained by T. T. E.-method comparing with the silver impregnation specimen of the same tissue

The specimen impregnated with silver by CAJAL's method (Fig. 1) presents numerous tigroids which give almost the same sizes and distribute homogeneously in the neuroplasm, but the figure of neurofibrils is not revealed. The nuclei of the satellite cells are arranged around the neuroplasm, but the satellite cell bodies are invisible.

The specimen of the same ganglia stained with T. T. E.-method presents the neuroplasm in heavy blue and the nucleus in light blue, but it lacks the granules of tigroid (Fig. 2). The nerve fibers present their courses with red tone (Fig. 2).

2) The AUERBACH'S plexus stained with T. T. E.-method

According to OKADA the human appendix has abundant nerve elements. The ganglia lie in many layers and scattered in the muscular walls. A ganglia consists of several nerve cells (Fig. 3, Fig. 4). Each ganglion has lightly red coloured cords, which present sometimes a faint network within them. The cords extend from a ganglia to another or toward submucous layer and communicate with the MEISSNER'S plexus (Fig. 5). Thus the cords give a tendency to form a large network.

3) MEISSNER's plexus.

Now, a ganglion in the MEISSNER's plexus is shown in Fig. 6. It is composed of 6 nerve cells and a cord spreads out of it toward a ganglion of AUERBACH's plexus. Within the red colour of the cord no fibrous structure is distinguishable except dark coloured small nuclei. Comparing these cords with the silver impregnated specimen (Fig. 3), one may easily find them to be SCHWANN's cell cords.

4) The capsule cells of nerve cells

As the nerve cells in the Fig. 4 show, the cell body are surrounded by a red coloured cord with dark nuclei scattered in it. The cord surrounding the nerve cell is the satellite cells without doubt, and the same red tone of satellite cells as SCHWANN's cell cords suggests that both consist of the same cell element. Fig. 7 shows a widened part of SCHWANN's cell cord, which may present in the silver impregnation specimen as the proliferation of the accessory cells.

In Fig. 8, there are two nerve cells in the MEISSNER's plexus. The upper smaller cell has an indistinct cell capsule and normal nerve cell body, while the lower one has no clear nerve cell figure. The nerve cell seems to be replaced with the cells of capsule. They seem to be socalled nodule residuelle or neuronocytolysis (HERZOG) (Fig. 8).

IV. DISCUSSION

The author stained the AUERBACH's plexus and the MEISSNER's plexus making use of FEYRTER's tartaric acid-thionin enclosure method and examined whether it can dill up the shortage of silver impregnation method of nerve structures or not. The distal nervous syncitium by JABONERO or the leading plasmodium by STOEHR is now regarded as SCHWANN's cell cord wrapping on the nerve fibers and the terminal reticulum by STOEHR without accompanied by nervous syncitium is denied by many authors (JABONERO, WEBER, HERZOG & etc.). The T. T. E.-method only reveals the SCHWANN's cells, capsule cells and accessory cells with red coloured homogeneous structures, but does not give clear figure of the nerve fibers nor of fibrils within them. Therefore, though it supports the hypothesis by DE CASTRO that the SCHWANN's cell, the capsule cell and the accessory cell are analogous ones with the oligodendroglia in the central nervous system, but it does not suggest anything on the relationship of them with nerve fibers. Concerning the correlation between SCHWANN's cell cord and nerve fibers the silver carbonate method by JABONERO may give far clear figures.

FEYRTER describes that the red tone of stained nerve element must be kind of lipoprotein. It is generally regarded with electron microscope that a non-myelinated fiber has single Axon-SCHWANN membrane and a myelinated nerve fiber has multilayered SCHWANN's lamella. The light red colour of non-myelinated fiber and the deep red tone of myelinated fiber prove that T. T. E.-method by FEYRTER stains the lipoprotein in the SCHWANN's cell elements especially of the SCHWANN's membrane in red in proportion to the content of the substance.

FEYRTER reports that T. T. E.-method presents the fine figures of nerve fibers

in the nervous syncitia, but the present author cannot confirm it.

Remembering YAMAMOTO's electron microscopic study that the fine nerve fibers in the far periphery have not an Axon-SCHWANN membrane around each fiber but have a single common SCHWANN's membrane surrounding them.

In such region, how can it be possible to reveal each nerve fiber only with the staining of SCHWANN's cells, capsule cells and accessory cells must be more reliable.

Otherwise the superiority of this method comparing with silver impregnation cannot be recognized.

V. CONCLUSION

FEYRTER's T. T. E.-method was used in staining the AUERBACH's plexus of human being and got following results.

1. The T. T. E.-method stains the SCHWANN's cell, capsule cell and the accessory cell in the same red tone, which suggests that these cells have a common cell origin.

2. The light red tone of non-myelinated nerve fibers and the deep red tone of myelinated nerve fibers are attributed that the fibers are stained with the aid of lipoprotein contained in Axon-SCHWANN membrane or SCHWANN's lamella.

3. This method of staining cannot fill up the silver impregnation method in many respects.

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和文抄録

酒石酸チオニン包埋染色法による人の虫垂の神経要素に関する神経組織学的研究

京都大学医学部外科学教室第2講座（指導：青柳安誠教授）
満田久和

自律神経の終末構造に於て終網を囲り種々な見解があるが，著者はFeyrterにより提唱された酒石酸チオニン包埋染色法を用い，人の虫垂に於ける自律神経の終末附近の構造を追求し，次の結果を得た．

1) T.T.E. 染色法はSchwann系細胞を染める特性を有し，同様の染色態度から神経細胞の被膜細胞及び副細胞等は共通の発生母体を有するものなることが推定された．

2) 無髄線維は淡く赤染し，有髄線維は濃く赤染するが，これはAxon-Schwann膜又はSchwannの髄板に含まれるLipoprotein(脂質と蛋白質の化合物)が染まるものと理解された．

3) この染色法は種々なる観点からみて鍍銀法の欠点を償い得るものとは考えられない．

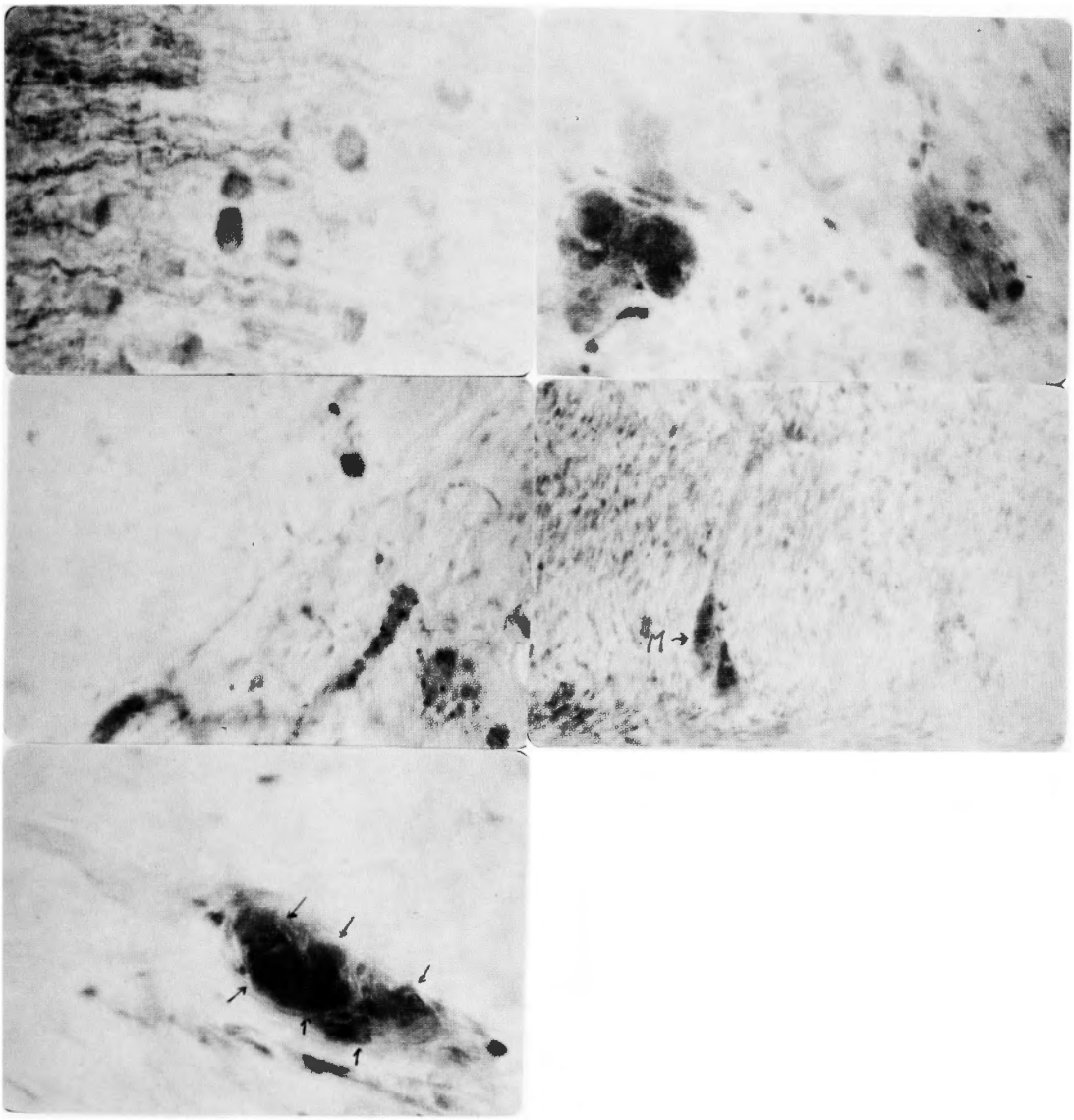

Fig. 2. The same specimen as Fig. 1 is stained with tartaric acid-thionin enclosure method. Nerve cells are stained blue and nerve fibers in (purpurish) red. Note the redish ring (capsule) surrounding a nerve cell.

Fig. 3. A small ganglion in AUERBACH's plexus. Nerve cells with a red coloured cord (accessory cells).

Fig. 4. A red coloured cord gives rise of a branch on the course. This is the SHWANN's cell cord with nerve fibers within it.

Fig. 5. A SCHWANN's cell cord running from AUERBACH's plex. communicates with the nerve cells in MEISSNER's plex. (M).

Fig. 6. Nerve cells in MEISSNER's plex. A SCHWANN's cell cord extend toward AUERBACH's plex.

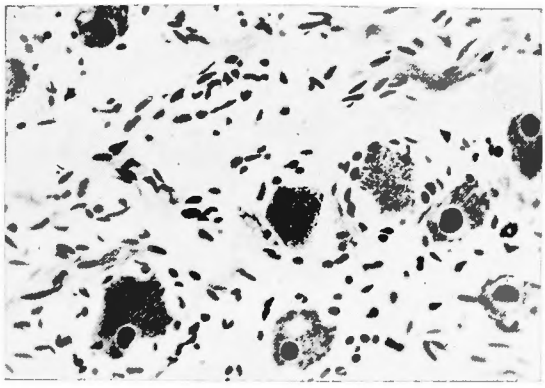

Fig. 1. Lumbar ganglion in the sympathetic trunk of human being. Silver impregnation with CAJAL's method.

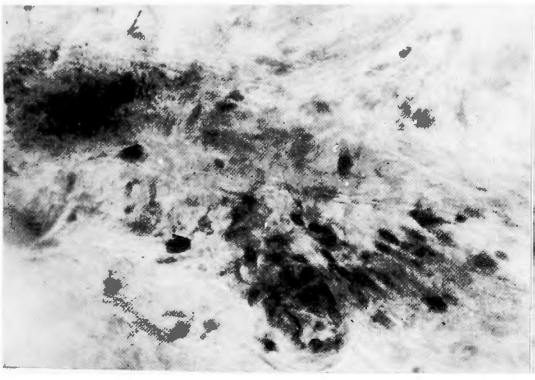

Fig. 7. The proliferated accessory cells are shown with a widened cord (↙).

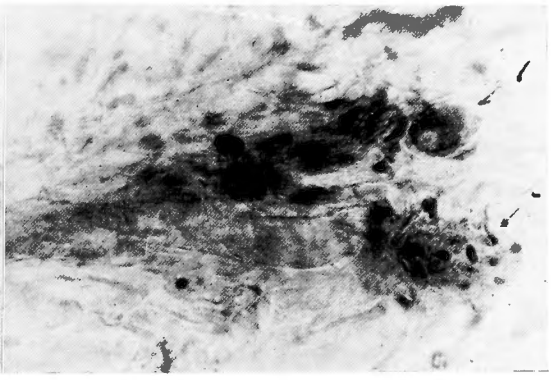

Fig. 8. 2 nerve cells in MEISSNER's plex.
1: normal nerve cell.
2: A nerve cell with proliferated satellite cells, i. e. neuronocyolysis or nodule residuelle.